VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

Paragraph beginning at line 16 of page 1, which has been amended as follows:

Since the invention of magnetic resonance imaging (MRI), a parallel technology of injectable chemicals called contrast agents has developed. Contrast agents play an important role in the practice of medicine in that they help produce more useful MRI images for diagnostic purposes. In particular, two classes of imaging agents have been developed and adopted in clinical practice. These are: low molecular weight gadolinium complexes such as Magnavist®; and colloidal iron oxides [such as Feridex I.V.® and Combidex®]. Neither of these two types of agents is ideal. Problems encountered with these agents are shown in Table 1, and include: expense of components; inefficiency of synthesis; loss of coating if sterilized by [during terminal sterilization (] autoclaving [)]; narrow range of organ uptake for purposes of imaging; [toxic] side-effects at doses in vast excess, for example, 100mg/kg body weight; restriction of use to either first pass or equilibrium dosing, and others that are described herein. Agents that overcome these problems, and that combine the properties of these two types of contrast agents, are highly desirable.

Paragraph beginning Table 1 on line 1 of page 2, which has been amended as follows:

Table 1. Comparison of ideal properties of MRI contrast agents with properties of low molecular weight gadolinium based contrast agents and colloidal iron oxides.

Properties of an ideal contrast agent	low molecular weight gadolinium	Colloidal iron oxides
Low production costs: efficient synthesis	Yes	No
Autoclavable without excipients	Yes	No
T1 agent	Yes	Sometimes
T2 agent	No	Yes
Non toxic at vast excess	Yes	No
Imaging vascular compartment at early phase (as a bolus administration)	No	No

and at a late stage (equilibrium phase)		
Multiple administration in single examination	No	No
Image of multiple target organs	Yes	Sometimes
Bolus injection	Yes	No
Low volume of injection	No	No
Iron source for anemia	No	Yes

Paragraph beginning at line 12 of page 3, which has been amended as follows:

In yet another embodiment, the invention provides a method of formulating an iron oxide complex coated with a reduced polysaccharide. This composition is for pharmacological use and the composition has decreased toxicity in comparison to an analogous iron oxide complex coated with native polysaccharide. The method of formulating such an iron oxide complex comprises: producing a reduced polysaccharide iron oxide complex, and sterilizing the complex by autoclaving. The formulation provides polysaccharide which was produced by reacting the polysaccharide with one of a reducing agent selected from the group consisting of a borohydride salt or hydrogen in the presence of an hydrogenation catalyst. The reduced polysaccharide iron oxide complex <u>has</u> [having] such decreased toxicity. In a further aspect of the method, the iron oxide is superparamagnetic.

Paragraph beginning at line 14 of page 11, which has been amended as follows:

Methods of preventing clumping of the colloid induced by heat stress that have no effect on coating dissociation have also been described. These methods generally include the use of excipients during the autoclaving process. Groman et al., U.S. Patent 4,827,945, and Lewis et al., U.S. Patent 5,055,288, both patents hereby incorporated herein by reference, use citrate to prevent clumping of the particles when the coating dissociates. [However, the use of citrate in high concentration in combination with heat can cause toxicity.] Groman et al., U.S. Patent 5,102,652, hereby incorporated herein by

reference, uses low molecular weight carbohydrates such as mannitol to prevent clumping during autoclaving. These excipients increase the cost and complexity of manufacturing the product, yet do not solve the problem of dissociation of the polymer from the iron particle.

Paragraph beginning at line 25 of page 12, which has been amended as follows:

A dextran can elicit a sometimes fatal anaphylactic response when administered intravenously (i.v.) in man (Briseid, G. et al., *Acta Pharmcol. Et Toxicol.*, 1980, 47:119-126; Hedin, H. et al., *Int. Arch. Allergy and Immunol.*, 1997:113:358-359). Related adverse reactions have been observed also on administration of magnetic dextran coated iron oxide colloids. Non-magnetic dextran coated iron oxide colloids that have utility as hematinics, particularly as an adjunct to erythropoietin treatment for end stage renal dialysis patients, may [can also] have [similar] side effects.

Paragraph beginning at line 4 of page 16, which has been amended as follows:

The term "colloid" as used in this specification and the accompanying claims shall include any macromolecule or particle having a size less than about 250 nm. The iron oxide polysaccharide colloids of the invention have substantially improved physical characteristics and manufacturability compared to previously described materials. Improved physical characteristics are evident in the ability of the colloid to withstand heat stress, as measured by subjecting the colloid to a temperature of 121°C for 30 minutes. Colloid particles made according to the invention show less evidence of polysaccharide dissociation under stress, remaining colloidal, and exhibiting no appreciable change in size. [An example of a colloid with an unstable polysaccharide coating includes Combidex®, which when subjected to heat stress, lost 43% of its dextran coating, and increased in particle diameter size from 21 nm to 587 nm; significant clumping of material was observed upon visual analysis. Another superparamagnetic iron oxide dextran colloid, Feridex®, prepared according to U.S. Patent 4,770,183, also exhibited increased particle size, as demonstrated by the inability of the heat treated

colloid to pass through a filter having a 0.8μm pore size, after a heat treatment comprising only 30 minutes at 121°C.]

Paragraph beginning at line 3 of page 17, which has been amended as follows:

The colloids that are an embodiment of the invention can be used as contrast agents for magnetic resonance imaging (MRI) or in other applications such as magnetic fractionation of cells, immunoassays, magnetically targeted drug delivery, and as therapeutic injectable iron supplements. These colloids are particularly suited to parenteral administration, because the final sterilization typically is autoclaving, a preferred method since it eliminates viability of all cellular life forms including bacterial spores, and viruses. Previous methods for making colloids required the addition of excipients such as citrate or low molecular weight polysaccharides as stabilizers during the autoclaving process (see U.S. Patent 4,827,945 and U.S. Patent 5,102,652), or avoided heat stress altogether by use of filter sterilization (see U.S. Patent 5,150,726). Thus, the embodiments of the present invention comprising the colloid compositions, provide utilities as significantly improved MRI contrast agents, and hematinic agents that are iron supplements. The improvements provided in these agents over prior art are found in the following facts demonstrated in the examples herein: that the agents which are embodiments of the present invention are heat sterilizable by autoclaving, and are thus optimized for long-term storage at ambient temperatures; that these agents do not require the addition of excipients for maintenance of stability during the sterilization or storage processes; that the agents are non-toxic to mammals including humans at higher doses; that an effective dose of the agents used for imaging is a smaller amount of material than the agents described in the art; and that the pharmacokinetics following administration are such that iterated successive doses administered after a brief interval after administration of a first dose can be used to obtain additional images during a single clinical visit and use of the imaging apparatus.

Paragraph beginning at line 22 of page 19, which has been amended as follows:

In Examples 52-53, the presence of symtoms of toxicity to rats at doses in vast excess of reduced and non-reduced (native) dextran coated USPIOs was determined, with response to an anaphylactic type reaction. The extent of the anaphylatic type reaction is determined by volume of paw edemia. Similar studies were performed using native, reduced, and carboxymethylated reduced dextrans. The result are summarized Tables 11-14.

Paragraph beginning at line 21 of page 45, which has been amended as follows:

Example 52. Toxicity studies in rats. Toxicity of reduced dextran, non-reduced dextran, and CMRD coated colloids administered in vast excess to [in] rats.

Paragraph beginning at line 18 of page 51, which has been amended as follows:

No adverse reactions attributable to administration of the composition were observed among the treated subjects at any dose, including the highest doses (4mg/kg). [For comparison, in clinical trials of Feridex I.V.®, approximately 2-3% of treated patients reported back pain, even though Feridex I.V.® and other comparable imaging products are administered in much smaller doses (e.g., 0.56 mg of iron/kg body weight) in order to minimize adverse events and obtain useful contrast. These data indicate that an effective dose of the CMRD coated USPIO particles of the invention is safer than an effective dose of a previously approved imaging agent, Feridex I.V.®]